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DATA EVALUATION REPORT

FLUAZINAM

STUDY TYPE: METABOLISM AND PHARMACOKINETICS – RAT [OPPTS 870.7485 (§85-1)]
MRID 44807233

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
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Task Order No. 99-51W

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DATA EVALUATION RECORD

STUDY TYPE: Metabolism and Pharmacokinetics- Rat [OPPTS 870.7485 (§85-1)

DP BARCODE: D258235
P.C. CODE: 129098

SUBMISSION CODE: S561478
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): IKF-1216; Non-radiolabled (99.6%); [^{14}C] Radiolabeled (98%)

SYNONYMS: 3-chloro-N-[3-chloro-2,6-dinitro(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2-pyridinamine; B1216, PP192.

CITATION: McClanahan, R. (1995). Study to Identify the Metabolites of IKF-1216 (Fluazinam) in Rats: Final Report. Ricerca, Inc. 7528 Auburn Road, P.O. Box 1000, Plainsville, OH 44077-1000. Study No. 92-1091, Doc. No. 5306-92-0191-AM-002. September 15,1995. MRID 44807233. Unpublished.

STUDY SPONSOR: Ishihara Sangyo Kaisha, Ltd. 10-30, Fujimi 2-chome, Chiyoda-ku, Tokyo 102, Japan.

EXECUTIVE SUMMARY: In a metabolism characterization study (MRID No. 44807233), Fluazinam (IKF-1216) was administered by gavage at single doses of 0.5 mg/kg or 50 mg/kg, or 14-day repeated doses of 0.5 mg/kg/day. In addition to nonlabeled IKF-1216 (lot no. T9002, 99.6% purity), [^{14}C]-IKF-1216 labeled on the phenyl moiety (lot. No. 93-5, purity 98%, sp. act. 57.3 mCi/mmol) or pyridyl moiety (lot. No. 93-90, 98% purity; sp. act. 66.2 mCi/mmol) were also administered in some studies to assess metabolic cleavage of the phenyl or pyridyl ring of the test material. Experimental groups were established for overall distribution/excretion assessment and for analysis of biliary secretion The metabolite profiles of urine, feces, and bile were examined and major metabolites were identified.

There were no treatment-related deaths in the rats. Overall recovery of the administered radioactivity (reported in MRID Nos. 43521006, 43521007, and 43521008 and evaluated in a separate DER) was acceptable (93.10-103.55%). Excretion via the urine was minor. AMPA mercapturate and DAPA, the major urinary metabolites, represented only 0.05-0.39% of the administered dose. Radioactivity in the feces represented most of the administered dose (88.78-100.03%) as determined by review of MRID Nos. 43521006, 43521007, and 43521008 and evaluated in a separate DER. Identified fecal metabolites, however, represented only 11.20-68.59% of the administered dose. For all dose groups, most of the fecal radioactivity appeared to reside with unextractable components in the post-extraction solids (PES). Further analysis of the PES components using base hydrolysis indicated that most of this radioactivity could be attributed to hydrolysis products of AMPA and DAPA. PES radioactivity was also greatest for the low-dose group which was consistent with the lower overall accounting of identified metabolites for this group. Approximately 20-25% of the aqueous phase of the fecal extraction was identified as a cysteine conjugate of DAPA and represented <1% of the administered dose. With the exception of the low-dose group, parent compound represented most of the identified radioactivity in the feces. AMPA

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and DAPA were identified in the feces from all dose groups but these metabolites never represented more than 5% of the administered dose (except for high-dose females rats where AMPA accounted for 10.22%).

DAPA glucuronide and AMPA mercapturate were the major biliary metabolites but represented <4% of the administered dose. Total biliary radioactivity, however represented 25-34% of the administered dose (MRID Nos. 43521006, 43521007, and 43521008 evaluated in a separate DER). Analysis of chromatograms indicated that numerous other metabolites were present in the bile but were individually of insufficient quantity to allow for characterization.

Metabolite profiles from administration of different label positions (pyridyl and phenyl) indicated that there was no metabolic cleavage of the ring structures. Minor quantitative differences in metabolite recovery were observed between genders but not of sufficient magnitude to suggest biologically relevant differences in the metabolism of IKF-1216.

This metabolism study is **Acceptable/Guideline.** When considered together with the previously submitted general metabolism studies on IKF-1216 (MRIDs 43521004 through 43521008 and MRID 43553001), the requirement for a general metabolism study in rats [OPPTS 870.7485 (§85-1)] is satisfied.

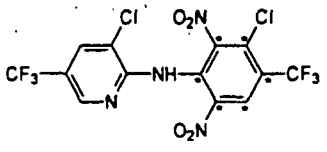
COMPLIANCE: Signed and dated GLP (p.1C and p. 3), Quality Assurance (p. 6), and Data Confidentiality statements (p. 1B) were provided in the study report. Flagging statements were not provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material

Radiolabeled
Name: [^{14}C]IKF-1216 (phenyl moiety label)
Description: yellowish, crystalline
Lot No.: 93-5
Purity: 98%
Sp. activity: 57.3 mCi/mmol (2.12 GBq/mmol)
Contaminants: none noted
Structure:



* = ^{14}C Label

Name: [^{14}C]IKF-1216 ([2,6-^{14}C]pyridyl moiety label)
Description: yellowish, crystalline
Lot No.: 93-90
Purity: 98%

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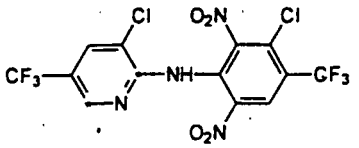
Sp. activity: 66.2 mCi/mmol (2.45 GBq/mmol)
Contaminants: none noted
Structure:



* = ^{14}C Label

Non-radiolabeled
Name: IKF-1216
Description: yellowish, crystalline
Lot No.: T9002
Purity: 99.6%
Stability:
Contaminants: none noted
CAS No.: 79622-59-6

2. Vehicle

The test article was administered in 0.75% methylcellulose. Vehicle control groups received equivalent volumes of methycellulose without the test material.

3. Test animals

The test animals used for this metabolite identification study were those of the in-life studies reported in MRID Nos. 435530-01 (Biliary excretion), 435210-06 (Metabolite identification; interim report), 435210-07 (Distribution and excretion - single oral dose), and 435210-08 (Distribution and excretion - repeated dosing)
Species: rat
Strain: Sprague Dawley Crl:CD®BR VAF/Plus®
Age and weight at study initiation:
(MRID No. 43553001) males: 10-11 weeks, 234-331 g; no females used
(MRID No. 43521006) animals from Exp. nos. 1, 5, and 6 used for samples
(MRID No. 43521007) males: 7-9 weeks, 172-222 g; females: 7-9 weeks, 148-192 g
(MRID No. 43521008) males: ≈5 weeks, 167-209 g; females: ≈5 weeks, 131-157 g
Source: Charles River Breeding Laboratories, Inc., 9801 Shaver Road, Portage, MI or 401 South New Hope Road, Raleigh, NC.
Housing: During experiments, animals were housed in Nalgene® metabolism cages
Environmental conditions:
Temperature: 65-78 °F
Humidity: 39-60%
Air changes: 10/hr
Photoperiod: 12 hr light/dark for all studies
Acclimation period: minimum of 5 days for all studies

4. Preparation of dosing solutions

Dosing suspensions were prepared as described for the aforementioned in-life studies described in MRID Nos. 43553001, 43553006, 43553007, and 43553008. Separate dose suspensions were prepared for the low and high doses. For the high dose, a homogeneous mixture of nonradiolabeled and labeled IKF-1216 was prepared by dissolving each in acetone, evaporating the acetone under nitrogen and reconstituting the residue in methylcellulose vehicle. For the low dose, only radiolabeled test material was used.

B. STUDY DESIGN AND METHODS

1. Group arrangements

The experiments described in the aforementioned reports (MRID Nos. 435530-01, 435210-06, 435210-07, and 435210-08) from which test samples were derived for this study are summarized in Table 1. Rats were assigned to the following treatment groups using a computer-generated random permutation program.

TABLE 1. Experimental Protocol

Study Type (Study No.)	Dose[a] (mg/kg)	Route of administration	No. of animals		Time of sacrifice
			Males	Females	
Biliary excretion; MRID No. 43553001	0.5 (low)[b,c] 50.0 (high)[b,c]	gavage gavage	7 6	0 0	48 hrs
Metabolite identification; MRID No. 43521006	0.5 (low)[b] 50.0 (high)[b]	gavage gavage	[b] [b]	[b] [b]	[b] [b]
Distribution/Excretion - single oral dose; MRID No. 43521007	0.5 (low) 50 (high)	gavage gavage	5 5	5 5	168 hrs 168 hrs
Distribution/Excretion - 14-day repeated oral dose; MRID No. 43521008	0.5 (low)[c]	gavage	5	5	24 or 168 hrs[d]

[a] ^{14}C(B)-IKF-1216 (Fluazinam) administered in 0.75 (w/v) methylcellulose (≈55-60 μCi/kg); controls (one rat/sex/dose) received equal volumes of vehicle only.
[b] These experiments used samples from Exp. nos. 1, 5, and 6 (MRID Nos. 435530-01, 435210-07, and 435210-08).
[c] Animals received nonlabeled test material for 14 consecutive days followed by single dose of radiolabeled chemical on day 15.
[d] Following 15-day treatment, sacrifice times indicate time after administration of radiolabeled test article.
Data taken from Table 1, p. 6, MRID Nos. 43553001, 43521004, 43521005, 43521006, 43251007, 43251008.

2. Dosing and sample collection

Rats were administered the test material by gavage in accordance with the test protocols shown in Table 1. For precision in dose determination, the dosing syringe and contents were weighed prior to dosing and immediately after dosing. The actual dose in dpm or mg was calculated by multiplying the weight difference by the dpm/g or mg/g, respectively. Control rats received only the methylcellulose vehicle. Dosing volumes were approximately 10-11 ml/kg. Vehicle controls were given equivalent volumes of methylcellulose without the test article. In MRID No. 43553001 (biliary excretion), the rats were fasted for 16 hours prior to

administration of the dose and returned to feed 4 hours after administration of the test compound.

Weights and/or volumes of all collected samples were recorded. The following samples were collected for metabolite analysis:

Urine – In the biliary excretion study (MRID No. 43553001), urine samples were collected at 0-6, 6-12, 12-24, and 24-48 hours. For the single dose distribution/excretion study (MRID No. 43521007), urine samples from each animal were collected continuously over dry ice at 6, 12, 12-14, 24-48, 48-72, 96-120, 120-144, and 144-168 hrs. Samples were frozen and stored in the dark until analysis. Duplicate aliquots were subjected to liquid scintillation counting (LSC); remainder of samples were used for metabolite analysis. For the repeated dose distribution/excretion study (MRID No. 43521008), urine was collected throughout the 14-day treatment for the nonlabeled test article and at 6, 12, 24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours after dosing with ^{14}C(B)-IKF-1216 (Fluazinam).

Feces – In the biliary excretion study (MRID No. 43553001), feces were collected over dry ice for 0-6, 6-12, 12-24, and 24-48 hr intervals. For the distribution/excretion studies, feces were collected over dry ice at the same schedule as urine (with exception of the 6-hr sample). Samples were frozen and stored in the dark until analysis. Fecal samples were homogenized and duplicate aliquots analyzed, following biological oxidation, by LSC for test article-derived radioactivity.

3. Sample preparation/analysis

Urine - Pooled urine samples were generated from both the single dose distribution/excretion study and the repeated dose study (0-12 or 0-48 hour collection times). Following centrifugation to remove sediment, the supernatants were concentrated and analyzed by HPLC.

Feces -For chromatographic analysis, fecal samples from a specific treatment group were pooled based on collection time. Collection included 24 and 48-hour sampling periods from the distribution/excretion studies and 24 and 48-hour samples from the biliary studies. Because of insufficient sample (due to their use in mass balance assessment for the original in-life studies), there were no pooled samples from the 12-hour period. The pooled samples were stirred to ensure homogeneity. As described in the Data Evaluation Report for the in-life studies, the pooled samples were sonicated, extracted with acetonitrile/H_2O, and partitioned with CH_2Cl_2 (dichloromethane) into organic and aqueous phases. The organic phase underwent rotary evaporation and subsequent HPLC/RAD analysis. The aqueous phase was concentrated and subjected to HPLC/RAD analysis.

Bile - Bile samples were pooled for the 6, 12, 24, and 48-hour collection periods from the in-life studies. Both phenyl and pyridyl labeled test material were used in the biliary elimination experiments. The percent of the administered dose in these samples ranged from 0.95 to 15.94%. The bile samples were centrifuged and the supernatant analyzed by HPLC. As described in the Data Evaluation Report for the in-life studies, DAPA glucuronide was isolated by repetitive injections into an HPLC and chromatographic system. AMPA mercapturate was isolated by progressive HPLC separation using varying solvents and gradient elutions (MeOH/water, EtOAc/water), and columns (C-18 packing and HPLC, silica

gel). Metabolites were identified by coelution with known standards for DAPA glucuronide, AMPA mercapturate, and the parent compound (IKF-1216). Base hydrolysis of bile using 1 N NaOH and comparison to HPLC retention for known AMPA mercapturate and DAPA glucuronide was used to confirm the presence of conjugated metabolites of Fluazinam.

Analytical techniques

High Performance Liquid Chromatography (HPLC): Both radioactivity detection (HPLC/RAD) by a scintillant cell and liquid scintillation counting (HPLC/LSC) of the HPLC fractions were utilized to quantify the percentage of radioactivity in the separated components. In addition to the HPLC system previously described for MRID 43521006, the following two systems were also used:

System H - Zorbax ODS 4.6 x 250 mm column with 5 micron particle size; 1.0 mL flow rate with UV detection at 254 nm in a Time-Resolved Liquid Scintillation Cell; 30-min gradient using Solvent A (1% acetonitrile in water ; 85 - 0%) and Solvent B (1% acetic acid in acteonitrile; 15 - 100%). System H was used for purification of metabolites in the aqueous fractions of fecal extracts.

System I - Zorbax ODS 4.6 x 250 mm column with 5 micron particle size; 1.0 mL flow rate with UV detection at 254 nm and lithium glass cell; 20-minute gradient using Solvent A (1% acetonitrile in water; 50 - 25%) and Solvent B (acetonitrile; 50 - 75%). System I was used for purification of metabolite reduction reaction products.

Liquid Scintillation Counting (LSC): LSC was performed using a Beckman LS6500 counter and Ultima Gold® or ScintiVerse®E liquid scintillation cocktail. Liquid samples (fecal extracts, HPLC effluent, urine and bile samples) were aliquoted by weight or volume and counted in duplicate or triplicate. Solid samples were oxidized and counted for a minimum of 2 minutes. Background was determined as 35-40 cpm. Disintegrations per minute were determined by an internal microprocessor and instrument resident quench curve.

Combustion and trapping efficiencies performed where appropriate (described in MRID 43521006), and method validation and instrument sensitivity were performed.

Mass Spectrometry (MS): Several systems involving MS were used for fractionation of the test material and analysis/identification of metabolites:

Gas Chromatography/Electron Ionization/Mass Spectra (GC/EI/MS) - Analyses were performed using a Finnigan 4510B quadrupole mass spectrometer interfaced with a Nova 4/C computer and appropriate software.

Thermospray Liquid Chromatography/Mass Spectrometry (TSP/LC/MS) - This system coupled liquid chromatography (Waters, Millipore Corp.) using a UV detector with a Vestec 201 or Finnigan SSQ710 thermospray LC/MS.

Ion-Spray Mass Spectrometry - A Sciex API III mass spectrometer was used.

Liquid Chromatography/Mass Spectrometry/Mass Spectrometry (LC/MS/MS) Fragmentation information on metabolites was obtained using a Sciex API III mass spectrometer connected in series with the liquid chromatograph.

Liquid Chromatography/Atmospheric Pressure Chemical Ionization/Mass Spectrometry (LC/APCI/MS) - A Waters liquid chromatograph using an Ultracarb 5 C8 column was used in conjunction with a Finnigan SSQ710 LC/MS equipped with an API/ESI source. The chromatograph system used a mobile phase of acetonitrile/water (65:35) containing 0.25% acetic acid at a flow rate of 0.2 mL/min. Both positive and negative ions were analyzed depending on the sample.

Liquid Chromatography/Electrospray Ionization/Mass Spectrometry (LC/ESI/MS) - LC coupled with electrospray ionization utilized a Waters LC system as described for LC/APCI/MS. The analyses also included passing of the eluent through an LC/MS equipped with an APCI/ES ion generating source.

Nuclear Magnetic Resonance (NMR): NMR was performed on a Varian Unity 600 NMR at an operating frequency of 600 MHz.

Degradation Procedures: Degradation of AMPA mercapturate, DAPA, fecal post-extraction solid samples, and bile were performed using base hydrolysis. Additionally, reduction of IKF-1216, AMPA, DAPA, and AMPA mercapturate was performed using 57% HI. Following HI reduction at 95-100°C for 2 hours, the samples were cooled, neutralized with sodium bicarbonate, and extracted with methylene chloride. The organic fraction was concentrated and analyzed by HPLC and liquid chromatography.

4. Statistics

Information regarding statistical analysis of metabolism data were not provided.

II. RESULTS

A. DISTRIBUTION OF METABOLITES

1. Accountability of identified metabolites

Total accountability (expressed as percent of administered dose) for metabolites identified in the various studies is shown in Table 2. Total metabolite production did not vary greatly between males and females. Fecal excretion was the major route of elimination and represented approximately 50-75% of the administered dose. Urinary metabolites accounted for less than 5% of the administered dose. Parent compound (IKF-1216) represented the majority of recovered radioactivity for all dose groups. Total accountability for the low-dose group was 29% less for males and 16% less for females than for the high-dose group. The amounts of parent compound (IKF-1216) appearing in the feces were also much lower for the low-dose group. These lower levels of parent compound suggests a relatively greater biotransformation at the lower dose but this is not supported by a corresponding increase in metabolite production.

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TABLE 2. Total Accountability (percent of dose) of Identified Metabolites						
Metabolite	Single High Dose (50 mg/kg)		Single Low Dose (0.5 mg/kg)		Repeated Low Dose (0.5 mg/kg)	
	Males	Females	Males	Females	Males	Females
IKF-1216 Feces Urine	45.13 –	54.93 –	7.64 –	2.13 –	27.47 –	36.83 –
AMPA Feces Urine	5.01 –	10.22 –	3.33 –	4.43 –	4.55 –	3.46 –
DAPA Feces Urine	3.36 0.07	3.07 0.18	4.52 –	4.64 –	1.72 0.05	0.99 0.23
DAPA glucuronide Feces Urine	– 0.00	– 0.00	– –	– –	– –	– –
AMPA mercapturate Feces Urine	– 0.15	– 0.19	– –	– –	– 0.08	– 0.39
Total	53.72	68.59	15.49	11.20	33.87	41.90
Total fecal radioactivity[a]	94.23	91.60	93.90	88.78	93.40	103.55

[a] Includes unextractable radioactivity from post-extraction soilds as reported in a separate DER evaluating MRID Nos. 43521006, 43521007, and 43521008.
Data taken from Tables 7-9, pp. 67-69, MRID 44807233.

Total accountability of identified metabolites from the biliary excretion studies using phenyl- or pyridyl-labeled test article is summarized in Table 3. As shown in the Table 3, AMPA mercapturate and DAPA glucuronide were major biliary metabolites of IKF-1216, although neither represented greater than 4% of the administered dose. Both AMPA mercapturate and DAPA glucuronide were also detected in the urine but at notably lower levels than for bile. Both of these metabolites were detected regardless of the label position on the test article but there was a notable quantitative difference between genders especially relative to urinary excretion of these conjugates. The study author noted that numerous other biliary metabolites occurred at levels too low for analysis. The presence of AMPA and DAPA in the feces of rats with biliary canulae indicated that gut microflora were also capable of forming these metabolites.

NOTE: AMPA is 4-chloro-N^2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]-3-nitro-5-(trifluoromethyl)-1,2-benzenediamine. See p. 11 of this DER for structure.

DAPA is 4-chloro-N^2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]-5-(trifluoromethyl)-1,2,3-benzenetriamine. See p. 11 of this DER for structure.

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TABLE 3. Total Accountability (percent of dose) of Identified Metabolites from Biliary Excretion Studies (0-48 hr samples)				
Metabolite	Phenyl label		Pyridyl label	
	Male	Female	Male	Female
IKF-1216				
Feces	24.87	45.42	27.59	35.64
AMPA				
Feces	3.57	5.69	4.09	5.07
DAPA				
Feces	2.49	7.46	3.68	3.36
Urine	0.07	0.18	0.48	0.43
DAPA glucuronide				
Urine	0.05	0.25	1.33	0.13
Bile	3.98	2.14	1.47	2.86
AMPA Mercapturate				
Urine	0.06	0.74	1.83	0.48
Bile	3.81	2.71	3.12	0.87
Total	38.90	64.59	43.59	48.84
Overall total[a]	62.82	64.59	68.24	71.10

[a] Includes estimated 12-hour fecal sample contribution determined by ratio of percent identified to total percent for 24- hour sample (ratio of 0.87 and 0.84 for phenyl and pyridyl, respectively).
Data taken from Table 17, p. 77, MRID 44807233.

B. METABOLITE CHARACTERIZATION STUDIES

1. Urinary metabolites

HPLC/RAD analysis of urine samples (0-24 hr pooled samples) from the distribution/excretion and repeated dose studies revealed three major metabolites and several minor metabolites. Based upon HPLC chromatograms, the major urinary metabolite profiles from the pyridyl and phenyl labels did not differ. Comparisons with known standards indicated that the major metabolites coeluted with AMPA mercapturate, DAPA glucuronide, and DAPA. Analysis of the chromatograms also revealed many minor metabolites (some as shoulders on the major peaks and others as distinct components) that could not be further characterized due to their minuscule concentrations.

2. Fecal metabolites

Both organic and aqueous phases from pooled 24 and 48-hr samples of fecal extracts were analyzed. HPLC analysis revealed parent compound, AMPA, and DAPA as the major components in the organic phase of the extracts for both the phenyl and pyridyl-labeled test article. Analysis of the aqueous phase of the extracts from feces of bile duct cannulated rats showed three components each representing no more than 1.52% of the administered dose. One component was identified as parent compound and the others were not identified. Label position did not result in a notable quantitative difference in the aqueous phase fractions. The

chromatogram for the aqueous phase metabolites from the distribution study varied somewhat from that for the aqueous phase biliary metabolites. The differences appeared to be primarily quantitative and possibly due to gut microflora activity. However, several polar metabolites representing very small fractions of the administered dose were identified in the aqueous phase extract from fecal samples from the high-dose rats. One of these was identified as a cysteine conjugate of DAPA which represented approximately 20-25% of the aqueous fraction. Because a substantial portion of the radioactivity associated with the feces resided with an unextractable residue (10-66%) in the post extraction solids (PES), additional effort was made to characterize this component by use of base (1N NaOH) hydrolysis to form hydrolysis products. Analysis of these hydrolysis products revealed that they were AMPA mercapturate and DAPA.

3. Biliary metabolites

Results of coelution experiments revealed that the major biliary metabolites were AMPA mercapturate and DAPA glucuronide. These metabolites represented less than 4% of the administered dose and both were formed regardless of label position. The chromatograms revealed many biliary metabolites but, with the exception of the AMPA mercapturate and DAPA glucuronide, they represented very small contributions to the administered dose.

A metabolic pathway proposed by the study author is shown in Figure 1.

III. DISCUSSION

A. DISCUSSION

The metabolism of orally administered Fluazinam (IKF-1216) was studied in male and female Sprague-Dawley rats. Single doses of 0.5 mg/kg or 50 mg/kg, or a 14-day repeated dose of 0.5 mg/kg/day were administered by gavage in 0.75% methylcellulose. In addition to nonlabeled IKF-1216 (lot no. T9002, 99.6% purity), [^{14}C]-IKF-1216 labeled on the phenyl moiety (lot. No. 93-5, purity 98%, sp. act. 57.3 mCi/mmol) or pyridyl moiety (lot. No. 93-90, 98% purity; sp. act. 66.2 mCi/mmol) were also administered in some studies to assess metabolic cleavage of the phenyl or pyridyl ring of the test material. In addition to experimental groups for distribution/excretion studies, the bile ducts of additional groups of both high and low-dose rats were cannulated. The metabolite profiles of urine, feces, and bile were examined and major metabolites were identified.

There were no treatment-related deaths in the rats. Overall recovery of the administered radioactivity (reported in MRID Nos. 43521006, 43521007, and 43521008 and evaluated in a separate DER) was acceptable (93.10-103.55%). Analysis of the aforementioned studies, revealed that urinary excretion was a minor route of elimination (1.36-4.32% of the administered dose). The current study identified AMPA mercapturate and DAPA as urinary metabolites but these metabolites represented only 0.05-0.39% of the administered dose. Radioactivity in the feces represented most of the administered dose (88.78-100.03% as determined by review of MRID Nos. 43521006, 43521007, and 43521008 and evaluated in a separate DER). Identified metabolites in the feces, however, represented only 11.20-68.59% of the administered dose. The radioactivity associated with identified metabolites was especially low (15.49 and 11.20% for low-dose males (15.49% of administered dose) and females (11.20% of administered dose).

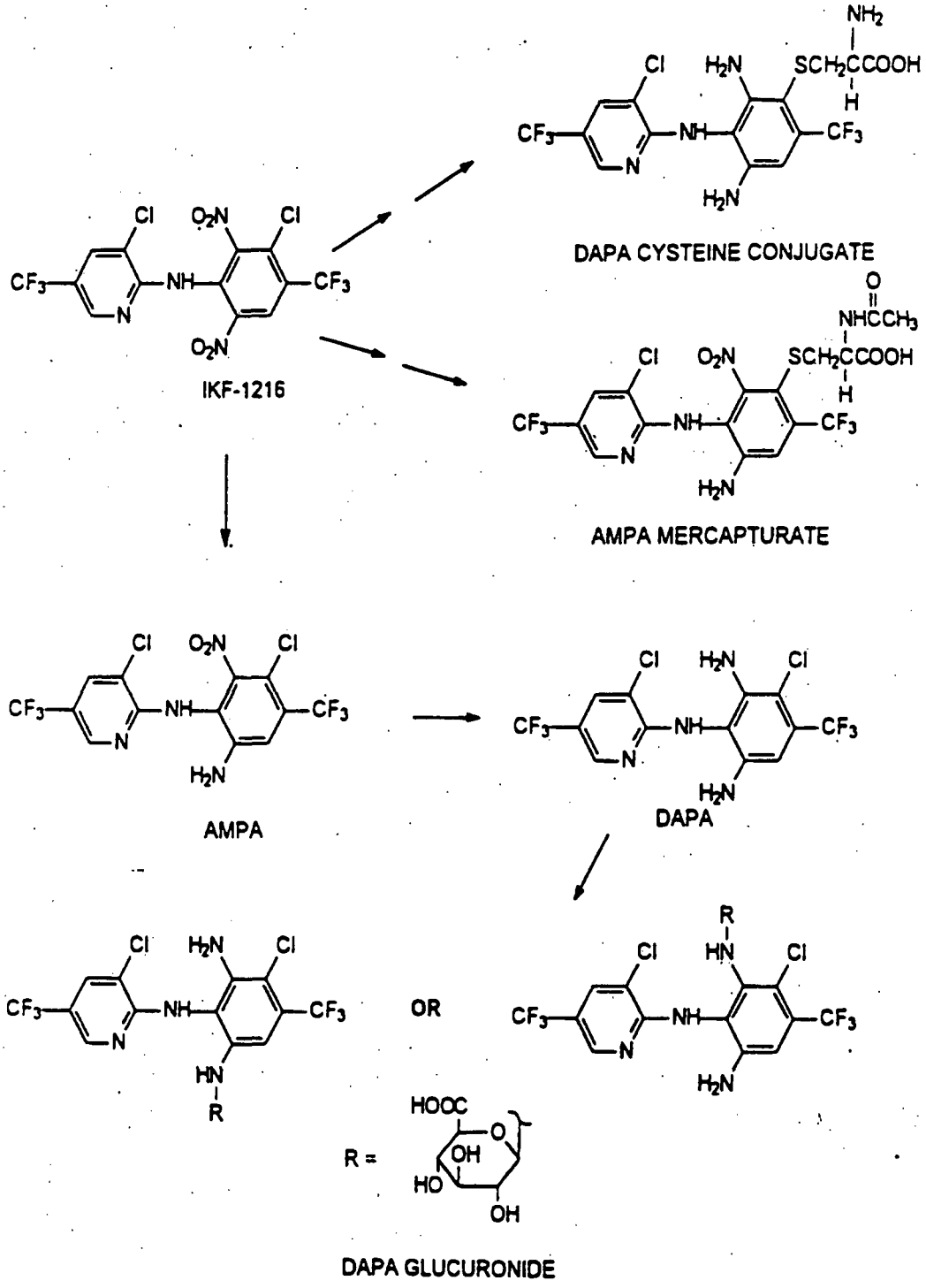

Figure 1. Proposed Metabolic Pathway of IKF-1216 in the Rat.
Source. Figure 37, p. 117, MRID 44807233.

Approximately 20-25% of the aqueous phase of the fecal extraction was identified as a cysteine conjugate of DAPA and represented <1% of the administered dose. For all dose groups, most of the fecal radioactivity appeared to reside as unextractable components in PES (post-extraction solids). Further analysis of the PES components using base hydrolysis indicated that most of this radioactivity could be attributed to hydrolysis products of AMPA and DAPA. PES radioactivity was also greatest for the low-dose group which is consistent with the lower overall accounting of identified metabolites for this group. With the exception of the low-dose group, parent compound represented most of the identified radioactivity in the feces. AMPA and DAPA were identified in the feces from all dose groups but these metabolites never represented more than 5% of the administered dose (except for high-dose females rats where AMPA accounted for 10.22%).

DAPA glucuronide and AMPA mercapturate were the major biliary metabolites but represented only <4% of the administered dose. Total biliary radioactivity, however represented 25-34% of the administered dose (MRID Nos. 43521006, 43521007, and 43521008 evaluated in a separate DER). Analysis of chromatograms indicated that numerous other metabolites were present in the bile but were individually of insufficient quantity to allow for characterization.

Similar metabolite profiles resulted from the administration of radiolabeled test material with different label positions (pyridyl and phenyl) indicating that there was no metabolic cleavage of the ring structures. Minor quantitative differences in metabolite recovery were observed between genders but not of sufficient magnitude to suggest biologically relevant differences in the metabolism of IKF-1216.

A metabolic pathway presented by the study author appears to be consistent with the available data.

This metabolism study is **Acceptable/Guideline.** When considered together with the previously submitted general metabolism studies on IKF-1216 (MRIDs 43521004 through 43521008 and MRID 43553001), the requirement for a general metabolism study in rats [OPPTS 870.7485 (§85-1)] is satisfied.

B. STUDY DEFICIENCIES

It appears uncertain as to whether or not the metabolite components in PES are, in fact, due to biliary contribution or the result of gut microflora activity. The study author suggests that because the PES radioactivity is similar to that of the bile, the components have similar origins. The reviewer questions if this is a valid statement. The fact that the PES radioactivity is greater at later collection times than at early collection times would appear to support the contention that gut microflora activity is responsible for the unextractable PES metabolites. Although this uncertainty does not negatively impact on the validity of the study or its conclusions, some additional discussion may be warranted especially in light of the fact that the PES radioactivity represents a notable portion of the administered dose in the low-dose group.

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